

**Amendments to the Specification:**

Please replace the paragraph beginning on page 1, line 3 of the specification with the following amended paragraph:

Related Applications

This application is the National Stage of International Application No. PCT/US00/26648, filed September 28, 2000, which claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application 60/157,216, filed October 1, 1999, the disclosures of which are herein incorporated in their entirety by reference.

Please replace the paragraph beginning on page 1, line 15 of the specification with the following amended paragraph:

The Wuschel protein, designated hereafter as WUS, plays a key role in the initiation and maintenance of the apical meristem, which contains a pool of pluripotent stem cells (Endrizzi et al., 1996, *Plant Journal* 10:967-979; Laux et al., 1996, *Development* *Development* 122:87-96; and Mayer et al., 1998, *Cell* 95:805-815). Arabidopsis plants mutant for the WUS gene contain stem cells that are misspecified and that appear to undergo differentiation. WUS encodes a novel homeodomain protein, which presumably functions as a transcriptional regulator (Mayer et al., 1998, *Cell* 95:805-815). The stem cell population of Arabidopsis shoot meristems is believed to be maintained by a regulatory loop between the CLAVATA (CLV) genes which promote organ initiation and the WUS gene which is required for stem cell identity, with the CLV genes repressing WUS at the transcript level, and WUS expression being sufficient to induce meristem cell identity and the expression of the stem cell marker CLV3 (Brand et al. (2000) *Science* 289:617-619; Schoof et al. (2000) *Cell* 100:635-644). Constitutive expression of WUS in Arabidopsis has

been recently shown to lead to adventitious shoot proliferation from leaves (*in planta*) (Laux, T., Talk Presented at the XVI International Botanical Congress Meeting, August 17, 1999, St. Louis, MO).

Please replace the paragraph beginning on page 10, line 4 of the specification with the following amended paragraph:

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without ~~effecting~~ affecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

Please replace the paragraph beginning on page 16, line 24 of the specification with the following amended paragraph:

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' ~~Non-coding~~ non-coding sequences encoding transcription termination signals may also

be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Please replace the paragraph beginning on page 19, line 27 of the specification with the following amended paragraph:

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95 96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325 332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077 1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) (1994) *Nat. Genet.* 7:22 28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Acids Res.* 17:6795 6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Please replace the paragraph beginning on page 26, line 15 of the specification with the following amended paragraph:

Sunflower meristem transformation is achieved by a protocol for direct DNA delivery by particle bombardment or a protocol involving a combination of DNA-free particle bombardment followed by use of *Agrobacterium* inoculation for DNA delivery as

described in Bidney et al. (supra). Sunflower line SMF3, described in Burrus et al. (1991, Plant Cell Rep. 10:161-166) is used. The explant source is dry sunflower seed that is imbibed and dissected into meristem explants. Seeds are dehulled and surface sterilized then placed in sterile petri plates on two layers of filter paper moistened with sterile distilled water for overnight imbibition in the dark at 26°C in a ~~Perival~~ Percival incubator. The next day, cotyledons and root radicle are removed and meristem explants transferred to 374E medium (MS salts, Shepard vitamins, 40 mg/l adenine sulfate, 3% sucrose, 0.5 mg/l 6 BAP, 0.25 mg/l IAA, 0.1 mg/l GA, pH 5.6, and 0.8% Phytagar). Explants are cultured for 24 hr on 374E medium in the dark at 26°C. Following this culture period, elongated primary leaves are removed to expose the apical meristem. The meristem explants are placed in the center of petri plates with 374M medium (374E with 1.2% Phytagar) in preparation for particle bombardment then back in the dark for another 24 hr period at 26°C.

Please replace the paragraph beginning on page 32, line 36 of the specification with the following amended paragraph:

The sample plates are ~~positioned~~ positioned 2 levels below the ~~steeping~~ stopping plate for bombardment in a DuPont Helium Particle Gun. All samples receive a single shot at 650 PSI, with a total of ten aliquots taken from each tube of prepared particles/DNA. As a control, embryos are bombarded with DNA containing the PAT selectable marker as described above without the gene of invention.

Please replace the paragraph beginning on page 34, line 17 of the specification with the following amended paragraph:

Meristem transformation protocols rely on the transformation of apical initials or cells that can become apical initials following reorganization due to injury or

selective pressure. The progenitors of these apical initials differentiate to form the tissues and organs of the mature plant (i.e., leaves, stems, ears, tassels, etc.). The meristems of most angiosperms are layered with each layer having its own set of initials. Normally in the shoot apex these layers rarely mix. In maize the outer layer of the apical meristem, the L1, differentiates to form the epidermis while descendents of cells in the inner layer, the L2, give rise to internal plant parts including the gametes. The initials in each of these layers are defined solely by position and can be replaced by adjacent cells if they are killed or compromised. Meristem transformation frequently targets a subset of the population of apical initials and the resulting plants are chimeric. If for example, 1 of 4 initials in the L1 layer of the meristem are transformed only  $\frac{1}{4}$  of epidermis would be transformed. Selective pressure can be used to enlarge sectors but this selection must be non-lethal since large groups of cells are required for meristem function and survival. Transformation of a meristem cell with a WUS sequence under the expression of a promoter active in the apical meristem (either meristem-specific or constitutive) would allow the transformed cells to re-direct the initiation of new apical initials driving the meristem towards homogeneity and minimizing the chimeric nature of the plant body. To demonstrate this, the WUS sequence is cloned into a cassette with a promoter that is active within the meristem (i.e. either a strong constitutive maize promoter such as the ubiquitin promoter including the first ubiquitin intron, or a promoter active in meristematic cells such as the maize histone, *cdc2* or actin promoter). Coleoptilar stage embryos are isolated and plated meristem-up on a high sucrose maturation medium (see Lowe et al., 1997, In Genetic Biotechnology and Breeding of Maize and Sorghum, AS Tsaftaris, ed., Royal Society of Chemistry, Cambridge, UK, pp94-97). The WUS expression cassette along with a reporter construct such as Ubi:GUS:pinII can then be co-delivered (preferably 24 hours after isolation) into the exposed apical dome using conventional particle gun transformation protocols. As a control, the WUS construct can be replaced with an

equivalent amount of pUC plasmid DNA. After a week to 10 days of culture on maturation medium the embryos can be transferred to a low sucrose hormone-free germination medium. Leaves from developing plants can be sacrificed for GUS staining. Transient expression of the WUS sequence in meristem cells, through formation of new apical initials, will result in broader sectors or completely transformed meristems increasing the ~~probably~~ probability of germ-line transformation. Integration and expression of the WUS sequence will impart a competitive advantage to expressing cells resulting in a progressive enlargement of the transgenic sector. Due to the WUS-induced maintenance of apical initials and growth of their transformed derivatives, they will supplant wild-type meristem cells as the plant continues to grow. The result will be both enlargement of transgenic sectors within a given cell layer (i.e. periclinal expansion) and into adjacent cell layers (i.e. anticlinal invasions). As cells expressing the WUS gene occupy an increasingly large proportion of the meristem, the frequency of transgene germline inheritance goes up accordingly. Using WUS in this manner to target meristems will increase transformation rates, relative to control treatments. Coleoptilar-stage embryos used as a source of meristems is used as an example, but other meristem sources could be used as well, for example immature inflorescences.

Please replace the paragraph beginning on page 36, line 11 of the specification with the following amended paragraph:

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with ~~fluorescent~~ fluorescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

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Reply to Office Action of October 18, 2006

Please insert the following Abstract on page 42, following the claim set that ends on page 41: